Improvement of a synthetic medium for *Dictyostelium discoideum*

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Abstract

Dictyostelium discoideum is of considerable interest as an expression system for the production of proteins of high value. The cultivation of this social amoeba is not as easy as with other common microbial expression systems. Wildtype strains grow on bacteria. Mutant strains growing on axenic media reach cell densities of $1\text{-}2\cdot10^7$ mL^{-1} when cultivated in commonly used complex media. A totally synthetic medium formulated by Franke and Kessin in 1977 [1] has become popular and allows cell densities of about $3\cdot10^7$ mL^{-1} to be obtained. This medium (FM) is being improved mainly on the basis of the analysis of limitations with respect to amino acids. With this improved synthetic medium (SIH) cell densities in the order of $5\text{-}6\cdot10^7$ mL^{-1} have been achieved.

Materials and methods

Chemical substances and media components

Yeast extract and bacto tryptone were purchased from Difco and casein peptone and D-glucose from Merck (Darmstadt, Germany). Asparagine was obtained from ICN, histidine from Senn Chemicals and other amino acids from Ajinomoto. Vitamins were purchased from Fluka except folic acid which was obtained from Serva. Dihydroxystreptomycin sulphate was purchased from Fluka and Geneticin (G-418) from Serva. All other chemicals were at least of analytical grade.

Media preparation

The composition of the complex axenic medium HL-5C is given in **Table 1**. The pH was adjusted to 6.3 prior to autoclaving the medium for 20 min at 121 °C. Water was obtained from a purification device Seralpur Pro 90CN.

FM and SIH medium were prepared on the basis of four solutions containing either amino acids, vitamins, salts, or trace elements. The composition of these solutions is given in **Table 2**. They may be stored at -20 °C for convenience. The amino acid solution was prepared four times concentrated, the vitamin solution 20 times concentrated, the salt solution 50 times concentrated, and the trace element solution 10'000 times concentrated. 250 mL of the amino acid solution, 50 mL of the vitamin solution, 20 mL of the salt solution and 0.1 mL of the trace element solution together with with 10 g glucose, phosphate salts and antibiotics (50 mg dihydroxystreptomycin sulphate and 5 mg geneticin) were filled up to 500 mL with water. The pH was adjusted to 6.5 by adding dilute HCl or NaOH solutions. Finally, the volume of the medium was brought to 1 litre by adding water. The medium was sterilised by filtering through a membrane filter system Sartobran 300 (Sartorius, Göttingen, Germany). The sterile medium can be stored at 4 °C for about 4 weeks.

Strain and cultivation systems

Dictyostelium discoideum (AX2) was cultivated in Erlenmeyer-shake flasks on a rotary shaker with an eccentricity of 25 mm and a rotational frequency of 150 min^{-1} at a temperature of 21 °C. Unless stated otherwise shake flasks of 500 mL were used filled with 50 mL medium. The cultivation experiments were started with an inoculation cell density of about 10^5 mL^{-1}.

Table 1 Composition of common complex axenic media for *Dictyostelium discoideum*

Ingredients	Media composition / g $litre^{-1}$			
Common names	A	AX	HL-5	HL-5C
Literature	[6]	[7]	[8]	[9]
Yeast extract	0.5	7.15	5	5
Proteose peptone	-	14.3	5	5
Bacto peptone	5	-	-	-
Casein peptone	-	-	-	2.5
Thiotone	-	-	5	-
Bacto-tryptone	-	-	-	2.5
Glucose	5	-	10	10
Maltose	-	18	-	-
Na_2HPO_4	-	0.49	0.6	0.35
KH_2PO_4	2.25	0.49	0.34	1.2
$K_2HPO_4 \cdot 12\ H_2O$	1.5	-	-	-
$MgSO_4 \cdot 7\ H_2O$	0.5	-	-	-
pH	6.3	6.7	6.5-6.7	6.3

Table 2 Composition of synthetic media for *Dictyostelium discoideum*

Substances	Media composition		
Common names	**HL-5***	**FM**	**SIH**
Literature	[1]	[1]	this article
Glucose / mmol litre^{-1}	> 56	56	56
- **Amino acids** / mmol litre^{-1}			
L-Arginine	4.4	3.3	3.3
L-Asparagine	—	2.3	2.3
L-Aspartic acid	6.9	**—**	**1.1**
L-Cysteine·HCl	0.58	**1.7**	**2.5**
Glycine	16.6	12.0	12.0
L-Glutamic acid	9.7	**3.4**	**3.7**
L-Histidine	1.6	1.4	1.4
L-Isoleucine	4.3	4.6	4.6
L-Leucine	7.2	6.9	6.9
L-Lysine·HCl	5.9	**4.9**	**8.5**
L-Methionine	1.9	**2.0**	**2.3**
L-Phenylalanine	3.1	**3.0**	**3.3**
L-Proline	> 6.3	7.0	7.0
L-Threonine	4.3	4.2	4.2
L-Tyrosine	2.5	—	—
L-Tryptophan	0.52	**1.0**	**1.7**
L-Valine	5.9	6.0	6.0
- **Vitamins** / mg litre^{-1}			
Biotin	0.023	0.020	0.020
Cyanocobalamin	0.005	0.005	0.005
Folic acid	0.12	0.20	0.20
Lipoic acid	—	0.4	0.4
Riboflavin	0.4	0.5	0.5
Thiamine·HCl	0.53	0.6	0.6
Calcium pantothenate	0.59	—	—
Pyridoxine·HCl	0.18	—	—
Choline chloride	30.0	—	—
Nicotinic acid	3.7	—	—
p-Aminobenzoic acid	0.13	—	—
- **Phosphate salts** / mmol litre^{-1}			
K_2HPO_4	5.0	**5.0**	**—**
KH_2PO_4	—	**—**	**8.82**
NaH_2PO_4	—	**—**	**2.47**
- **Salts** / mmol litre^{-1}			
NaOH	7.0	**2.0**	**—**
NaCl	6.0	—	—
$NaHCO_3$	—	0.2	0.2
NH_4Cl	—	1.0	1.0
$CaCl_2$	0.31	0.02	0.02
$FeCl_3$	0.20	0.10	0.10
$MgCl_2$	0.49	0.40	0.40
- **Trace elements** / μmol litre^{-1}			
Na_2EDTA	—	13	13
H_3BO_3	—	1.8	1.8
$CoCl_2$	—	0.7	0.7
$CuSO_4$	6.4	0.6	0.6
$(NH_4)_6Mo_7O_{24}$	—	0.08	0.08
$MnCl_2$	1.4	2.6	2.6
$ZnSO_4$	13	8	8

* Analysed after hydrolysis

Comments:

This article should appear in the near future in Process Biochemistry. It is already available through SCIENCE@DIRECT.

One other Dicty-paper appeared recently:

- U. Beshay, K. Friehs, A.E.-M. Azzam, E. Flaschel : Cultivation of *Dictyostelium discoideum* in immobilized form by colonization of porous supports. *Process Biochem. 38* (2003) 1521-1529

Some other Dicty-papers are in print at the moment:

- M. Stephan, U. Beshay, K. Friehs, E. Flaschel: Influence of medium composition on growth behaviour of *Dictyostelium discoideum* for cultivation on axenic media. *Proc. Biochem.*
- U. Beshay, K. Friehs, A.E.-M. Azzam, E. Flaschel : Analysis of the behaviour of *Dictyostelium discoideum* in immobilized state by means of continuous cultivation. *Bioproc. Biosyst. Eng.*
- S.-I. Han, K. Friehs, E. Flaschel : Cultivation of *Dictyostelium discoideum* on an improved synthetic medium in a conventional bioreactor. *Proc. Biochem*.
- Y. Lu, U. Beshay, K. Friehs, E. Flaschel : Mass production of *Dictyostelium discoideum* in homogeneous and heterogeneous cultivation systems. *Proc. Biochem*.
- Y. Lu, J.C. Knol, M.H.K. Linskens, K. Friehs, P.J.M. van Haastert, E. Flaschel : Production of the soluble human Fas ligand by means of *Dictyostelium discoideum* cultivated on a synthetic medium. J. Biotechnol.